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Chlamydial abortion was first described by Greig[1] in 1936 and named enzootic abortion of ewes (EAE). At that time he suggested that it was the result of environmental factors such as dietary deficiency. It was not until 1950 that Stamp and colleagues[2] demonstrated that it was an infectious condition caused by an organism of the 'psittacosis-lymphogranuloma venereum group'. In the mid 1960s specific phenotypic traits of the chlamydial strains were identified (sulphadiazine sensitivity and glycogen accumulation), which together with inclusion morphology became the basis by which the strains were differentiated into the two species *Chlamydia trachomatis* and *C. psittaci*[3]. The *C. psittaci* group consisted of strains from a wide variety of animal sources, including that responsible for EAE, while the *C. trachomatis* group consisted of strains from human sources. The development of DNA-based classification methods, particularly DNA–DNA reassociation studies[4,5], in the 1980s led to the designation of two additional species, *C. pneumoniae*[6] and *C. pecorum*[7]. These studies also supported the classification of chlamydial strains into eight species groups, with a ninth identified in 1993[8], highlighting the need for a revision of chlamydial taxonomy. In 1999 Everett and colleagues[9] proposed a reclassification of the order Chlamydiales and its taxa based mainly on phylogenetic analyses of the 16*S* and 23*S* rRNA genes, but also on corroborating genetic and phenotypic information. A summary of this reclassification is shown in Table 1. The family Chlamydiaceae, which previously had only one genus Chlamydia, has been divided into two genera, Chlamydia and Chlamydophila. Within these genera five new species, in addition to the existing four, have been proposed. The organism responsible for ovine abortion, which was previously classified as serotype 1 *C. psittaci*, has been given species status and named *Chlamydophila abortus*.

Table 1 **Reclassification of the family**

	Previous classification	***Revised classification (Ref. 9)***	
Order	Chlamydiales	Chlamydiales	
Family	Chlamydiaceae	Chlamydiaceae	
Genus	Chlamydia	Chlamydia	Chlamydophila
Species	*C. trachomatis*	*C. trachomatis*	
		C. muridarum	
		C. suis	
	C. pneumoniae		*C. pneumoniae*
	C. psittaci		*C. psittaci*
			C. abortus
			C. felis
			C. caviae
	C. pecorum		*C. pecorum*

HUMAN INFECTION

Although few human cases occur annually, the danger to the pregnant woman and her developing fetus from exposure to *C. abortus* is considerable[10,11]. In most cases of *C. abortus*-induced human abortion there has been a direct association with exposure to contaminated sheep and goats. The outcome of human infection in the first trimester of pregnancy is likely to be spontaneous abortion, whereas later infection causes stillbirths or preterm labour[10]. Therefore, pregnant women must avoid all involvement with lambing ewes and lambs and should not handle contaminated clothing from those working with these animals. Immunocompromised people should also take great care to avoid contact with potential sources of infection at lambing time.

OVINE INFECTIONS

Although in many countries *C. abortus* infection is troublesome in ruminants and pigs, in the UK the disease principally occurs in sheep and goats. When chlamydial abortion occurs in a flock, stillborn lambs may be produced one to two weeks before the expected start of lambing although affected ewes may have a vulval discharge and show behavioural changes for up to 48 hours before this. The aborted lamb may look normal or show a degree of subcutaneous oedema. The placental membranes appear thickened and reddish-yellow, and a dirty pink infectious

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vaginal exudate may be noted for a further seven to ten days[12]. Subsequent contamination of the environment can act as a source of infection for susceptible female sheep as well as human beings. In sheep the primary infection probably becomes established first in the tonsil, from which it is disseminated by blood or lymph to other organs[13]. In non-pregnant animals infection becomes established as a latent infection, possibly in lymphoid tissue[14], in a process that can be mediated by cytokines[15].

LATENT INFECTION

The pro-inflammatory cytokine interferon-gamma (IFN-γ) is produced by sheep in response to challenge with *C. abortus*. Also, recombinant IFN-γ can restrict the growth of the organism in ovine cells in a dose-dependent manner that can be reversed by the addition of tryptophan[16]. Tryptophan degradation, as a result of induction of the enzyme indolamine 2,3-dioxygenase (IDO), is a common feature in many cell types treated with IFN-γ[15]. IFN-γ causes *C. abortus* infection in sheep cells *in vitro* to become latent in a manner that may mirror the situation in non-pregnant sheep *in vivo*[17]. IFN-γ (predominantly a T-cell product) has also been shown to have abortifacient properties in itself, so it is no surprise to find that there is a shift away from IFN-γ production during pregnancy, particularly at the maternofetal interface[18]. However, Munn and co-workers report that human and mouse trophoblast cells constitutively express IDO, and that limitation of tryptophan concentrations is important not only for controlling pathogen growth but also for mediation of peripheral T cell tolerance and maternal acceptance of the fetal allograft[19,20]. Further investigation is required to clarify the relationship between T cells, IFN-γ, IDO and *C. abortus*, since immune control of the organism seems weaker in pregnant than non-pregnant ewes.

PREGNANCY AND IMMUNITY

The factors that regulate immune recognition in mammals are highly complex and the triggers that switch the immune system 'on' (reactivity) or 'off' (anergy, tolerance) are the subject of continuing debate and research[21]. One fundamental principle of immunology states that the immune system is educated to discriminate between 'foreign' material (non-self) and that which is 'not foreign' (self) and react accordingly[22]. However, this is clearly not a hard-and-fast rule; for example, non-self is not rejected in pregnancy. Therefore, perhaps what is important is the 'appropriateness' and nature of immune reactivity. The tolerance of the maternal immune system to the semi-allogeneic fetus, carrying paternal antigens, has prompted hypotheses from immunologists for half a century[23]. Many mechanisms, it seems, combine to the success of pregnancy in outbred populations[24]. One mechanism in particular appears to be the down-regulation of certain cytokines such as IFN-γ, tumour necrosis factor-alpha (TNF-α) and interleukin-2 at the maternofetal interface (and possibly also in the maternal periphery) that are dangerous to the fetus[18]. However, this in itself may then make the fetus vulnerable to pathogens such as *C. abortus* that are controlled by host proinflammatory immune mechanisms, should they manage to invade the placenta.

PATHOGENESIS

In latently infected ewes the organism is undetectable by any means including serology[25]. During a subsequent pregnancy, it is thought that immune modulation allows chlamydial multiplication and an intermittent low-grade chlamydaemia that in turn initiates placental infection. The gestation period in sheep is around 143 days and placentation is cotyledonary, non-deciduate and epitheliochorial[26]. At around 60 days, maternal haematomata develop at the maternofetal interface in the hilus of each placentome. The hilar chorionic epithelial cells (trophoblast cells) are the first to be invaded by *C. abortus*. Athough it is tempting to conclude that the leaking of maternal blood into this region permits transmission of infection from mother to fetus, no pathological changes appear until after 90 days' gestation[27]. Thus, factors operate at this stage to release *C. abortus* from its state of suppression and permit the colonization of fetal placental cells[25]. Following establishment of infection in chorionic epithelial cells in the hilus of each of several placentomes, infection spreads out centrifugally into the surrounding intercotyledonary membranes where the resultant chorionic epithelial damage, oedema and inflammation give rise to the characteristic thickened placental membranes seen at the time of abortion. Ewes that become infected for the first time while pregnant may abort in the same pregnancy and so not develop latency[28].

The specific mechanisms responsible for abortion are unclear but the likely underlying cause is destruction of the chorionic epithelium. Progesterone, vital to the maintenance of normal pregnancy, is produced in the latter part of the ovine pregnancy by chorionic epithelial cells and interacts with oestradiol and prostaglandin in control of the onset of lambing. Levels of these three hormones are affected in a placental chlamydial infection and may therefore trigger fetal expulsion[29,30]. Maternal antibody titres to *C. abortus*, which remain low until after abortion (after which they rise), coincide with the development of protective immunity. Thus in sheep both humoral and cell-mediated mechanisms come into play[31], although the latter is of particular importance[15].

CONTROL MEASURES

If active chlamydial infection is thought to be present in a flock of pregnant ewes, treatment is an option. Long-acting oxytetracycline will reduce the severity of infection[32,33] and for best effect it should be given as soon after 95 days' gestation as possible, when placental infection may have begun, and a second injection two weeks later will further reduce losses. However, some ewes will still abort and many may still be infectious at lambing time. In general the use of antibiotics in this way should be reserved for exceptional circumstances, it being more desirable to control infection by management and vaccination. Management should aim to create and maintain a flock free of infection. This is best achieved if flocks are 'closed' and all replacement stock is obtained from farms known to be free of chlamydial infection. In the UK 'EAE accredited' flocks (members of the Premium Health Scheme run by the Scottish Agricultural Colleges' Veterinary Services) are a safe source. In many circumstances this strategy is impracticable and vaccination is the best approach. Non-pregnant healthy ewes can be vaccinated with one of the three currently available preparations, at any time until the four-week period before tupping. Thus sheep should be vaccinated in the first year after infection is first diagnosed in a flock and this should be repeated after three years, or sooner in heavily infected flocks. Sheep entering the flock for the first time should also be vaccinated.

Vaccine development

Field trials of a vaccine for ovine chlamydial abortion were begun as soon as its infectious nature was established[34]. Protective immunity was shown to be induced in sheep with a vaccine consisting of *C. abortus* grown in fertile hens' eggs and subsequently inactivated and incorporated with an oily adjuvant[35]. Of the three vaccines currently available in the UK, two consist of an attenuated strain of *C. psittaci* (Enzovax, Intervet, UK; Tecvax Chlamydia vaccine; Vétoquinol, UK) while the third is an inactivated preparation (Mydiavac, Novartis Animal Health, UK).

Although these vaccines offer adequate protection, improvements are necessary to avoid the problems associated with bulk chlamydial growth and purification, and because two of the preparations contain live organisms while the third relies on an oily adjuvant that may cause local inflammation. This requires a different approach to vaccine design involving the use of recombinant DNA technology to identify chlamydial antigens that can be used, as recombinant proteins or peptides, in subunit or multicomponent vaccines. Furthermore, the next generation of chlamydial vaccines will depend not only on identification of relevant antigens but also on ensuring that the antigens are correctly processed and presented to the immune system so that they stimulate the necessary protective immune response.

Vaccine research has largely focused on the predominant protein present in the outer cell membrane (OCM) of Chlamydia, the major outer membrane protein (MOMP). Experimental vaccines consisting of OCM preparations of *C. abortus*, of which MOMP constituted 90% of the protein content, afforded a high degree of protection from EAE, suggesting that MOMP was a major protective antigen[36]. This was further supported by studies with monoclonal antibodies to MOMP that were shown to prevent infection both *in vivo* and *in vitro*[37], and by MOMP peptide studies that identified protective T-cell epitopes[38]. However, vaccine studies to examine the efficacies of various forms of recombinant MOMP against experimental infection have been disappointing[39]. Although some protection was observed, the efficacies were variable and never as good as with whole organism and OCM-based preparations. There are two probable explanations for this, which are equally likely. The first is that the conformation of native MOMP, which is similar to that of other classic bacterial porin proteins[40,41], is a crucial factor for eliciting the correct protective immune responses. The second is that antigens additional to MOMP are required for good protection. Indeed, a group of highly immunogenic proteins with molecular masses of 90–95 kDa have been identified in the highly protective OCM preparation[42], and a monoclonal antibody to one of these has been shown to reduce chlamydial infectivity by 60%[43]. The genes coding for these proteins, referred to as the polymorphic outer membrane protein (POMP) or OMP90 family, have been cloned and sequenced[42] and at least one of the proteins has been shown to be surface exposed[44]. Although the function of the POMP proteins is unknown, they are currently attracting great interest primarily because genes encoding 9 and 21 orthologous proteins, respectively, have since been identified in both *C. trachomatis*[45,46] and *C. pneumoniae*[46,47]. The role of these proteins in protection is being investigated.

Another exciting and relatively new area of investigation is that of genetic or nucleic acid vaccination (also known as DNA vaccination). Major advantages of DNA vaccination, over the more conventional approaches, are that it more closely mimics natural infection, it induces good immunological memory, neonatal immunization is possible, there are no injection site reactions, and they are safer, with no possibility of contamination with adventitious agents[48]. Furthermore, DNA vaccines are easy and cheap to produce and are very stable. DNA vaccination induces both cellular and humoral immune responses, although crucially it is more consistent in inducing cellular responses, which are considered essential for the resolution of chlamydial infection[49]. Importantly, the immune response can be modulated to ensure that the most effective protective

responses are generated. This can be achieved through plasmid construction, method of delivery and route of immunization, by coadministration with costimulatory molecules, such as cytokines and chemokines, and by the inclusion of immunostimulatory sequences that enhance cellular responses[48,50,51].

DNA vaccines evoke a protective immune response to Chlamydiaceae in various animal model systems[52–57]. In particular, Murdin *et al.*[58] recently described the use of a DNA immunization strategy to identify protective antigens by screening selected open-reading frames from the *C. pneumoniae* genome. The identification of protective antigens by this approach is a significant step towards the development of a subunit vaccine and demonstrates the usefulness of DNA vaccination for determining the protective efficacy of other chlamydial genes/antigens.

CONCLUSIONS

The development of these improved vaccines will not only be of economic importance for farmers but will also reduce contamination of the environment at lambing time. This will in turn reduce the potential for *C. abortus* to trigger human infections.

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